DMRT1/Dmrt1, the Sex Determining or Sex Differentiating Gene in Vertebrata

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Although the phenomenon of sexual dimorphism is widespread in vertebrates, the molecular mechanism of sex-determination is not the same across animal phyla, in contrast to other areas of developmental biology. Recent extensive studies, however, have given proof of evolutionarily conserved function in genes which share a novel DNA binding DM domain, primarily identified in two invertebrate sex regulatory genes: doublesex of Drosophila melanogaster and mab-3 of Caenorhabditis elegans. Their mammalian autosomal homologue, DMRT1, first isolated in humans, was further discovered in genomes of various vertebrate species and appears to be involved in similar aspects of sexual development. Its precise role is still speculated, thus identification of sex reversal mutations, functional studies as well as determination of the sex-specific expression profile during embryogenesis are still being undertaken. Is this a sex determining rather than a sex differentiating gene? Is it involved in a dosage-sensitive mechanism? On what level does it function in the hierarchy of the sexual regulatory gene cascade? Recent results are discussed in this paper.

Key words: DMRT1, Dmrt1, sex determination, sex differentiation, vertebrate sexual development.

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The mechanisms leading to the development of sexual dimorphism vary remarkably between animal species, not only between phyla, but even in a single phylum. Vertebrates reflect these differences on the level of both switch mechanisms used in sex determination and the genes responding to these switch signals and involved in molecular sex regulatory pathways. Primary sex-determining signals can be either genetic or environmental. The known genetic signal is the presence of the Y chromosome in the heterogametic XY male sex in mammals and some fish or the as yet unknown genetic factor in the heterogametic ZW female sex in birds and some reptiles. The initial environmental cues include temperature (turtles and alligators), social factors (some fish) and population density (ZARKOWER 2001). Despite this diversity, all these sex-determining systems control the fate of bipotential embryonic gonads, i.e. they determine whether the gonads undergo testis or ovary development. Gonads do not only contain germ cells but also secrete hormones that conscript the body into sexual differentiation. Several genes are required for gonadal sexual development, the process of which might be divided into three steps: initial formation of the sexually indifferent gonads, the critical sex-determining period and gonad differentiation after sex determination.

The cascade of sex regulatory genes that act during gonadogenesis is best established in mammals (Fig. 1). The crucial gene for primary sex determination in most eutherian mammals is SRY/Sry (the sex-determining region on the Y chromosome), the heterosomal dominant male-determining locus which is responsible for testis formation from ambiguous XY gonads (SINCLAIR et al. 1990; KOOPMAN et al. 1991). This sufficient testis-determining factor seems not to be highly conserved among vertebrates due to its absence in non-mammalian animals. In birds, where sex is genetically determined (ZZ/ZW system), the heterosomal dominant male-determining locus which is responsible for testis formation from ambiguous XY gonads (SINCLAIR et al. 1990; KOOPMAN et al. 1991). This sufficient testis-determining factor seems not to be highly conserved among vertebrates due to its absence in non-mammalian animals. In birds, where sex is genetically determined (ZZ/ZW system), it still remains unknown whether the primary switch gene is a W-linked female dominant factor or a dosage-sensitive gene residing on the Z chromosome and triggering testis differentiation.

The autosomal gene SOX9/Sox9 (Sry-box containing gene 9), related to SRY/Sry, is postulated to be the immediate downstream target of SRY/Sry in
mammals and both SRY/Sry and SOX9/Sox9 are necessary for male sex determination in this animal taxon (KOOPMAN 1999). However, unlike SRY/Sry, SOX9/Sox9 plays an important role in male sexual development not only in mammals but also in birds (KENT et al., 1996) and reptiles (SPOTILA et al., 1998). Although it is upregulated in these vertebrate species, the expression of avian and reptilian Sox9 occurs after the onset of sexual differentiation, thus these homologues cannot be considered as primary sex-determining genes.

Do the vertebrates that lack Sry contain other switch gene(s) necessary for testis development during the critical sex determining period? Do mammals require other gene(s) for the unaffected developmental mechanism determining sexual dimorphism? This review indicates a new candidate gene that partly fills the gap in some vertebrate molecular sex-determining pathways.

DMRT1, a highly conserved gene in Vertebrata

Among genes involved in sexual development, DMRT1 (doublesex and mab-3 related transcription factor 1) is the only one found to date to be so far conserved between phyla. This evolutionary conservation refers not only to its nucleotide and protein sequence similarity, but also to its function. DMRT1 contains the strongly conserved DM domain, a new zinc finger-like DNA-binding motif, first identified in the doublesex gene in Drosophila melanogaster (ERDMAN & BURTIS 1993) and further in the mab-3 gene in Caenorhabditis elegans (RAYMOND et al., 1998). The role of these two invertebrate downstream sex regulatory genes in somatic sex determination and differentiation is well characterized (BURTIS & BAKER 1989; SHEN & HODGKIN 1988). Both genes control analogous aspects of sexual development: regulation of yolk protein gene transcription (YI & ZARKOWER 1999), differentiation of male-specific sense organs (SHEN & HODGKIN 1988, Yi et al. 2000) and mediation of male mating behaviour (Yi et al. 2000). Furthermore, they can be functionally interchangeable in vivo (RAYMOND et al., 1998). DMRT1, a human male regulatory gene related to invertebrate dsx and mab-3, is the first DM domain putative transcription factor discovered in a vertebrate genome (RAYMOND et al., 1998). The genomic organization of the gene (RAYMOND et al., 1999a), chromosomal mapping studies (RAYMOND et al., 1998), cytogenetic diagnosis and mutational screening of patients with sexual abnormalities (RAYMOND et al. 1999a; MUROYA et al. 2000; ÖUNAP et al. 2004) and analysis of expression profiles of the gene in both adult tissues (RAYMOND et al. 1998), and embryo sections (MONIOT et al. 2000), have provided significant data on the role of human DMRT1 in male sexual development and
its homology to sex regulatory genes in Nematoda and Antropoda. To better understanding the function of DMRT1 in the process of testicular differentiation in humans and to support its evolutionarily conserved status in the animal kingdom, these studies have been extended to other vertebrate species. Until now Dmrt1 has been identified from mammals (mouse, rat), birds (chicken), reptiles (alligator, turtle, lizard), amphibians (frog) and fish (Raymond et al. 1999b; Chen & Heckert 2001; Shan et al. 2000; Smith et al. 1999; Kettlewell et al. 2000; Torres-Maldonado et al. 2002; Murdock & Wibbels 2003; Sreenivasulu et al. 2002; Shibata et al. 2002; Brunner et al. 2001; Matsuda et al. 2002; Marchand et al. 2000; Guan et al. 2000; Guo et al. 2005). Sequence comparisons of various vertebrate Dmrt1 genes with human and invertebrate homologues and their chromosomal locations and embryonic expression profiles may indicate an implication in the first universal mechanism of sex determination across animal phyla.

All of these orthologues exhibit a high level of sequence identity with human DMRT1 (from 98% in mice to about 87% in fish at the amino acid level), but only inside a single DM domain that is present near the N-terminus. Outside the DM domain there is also another conserved proline/serine rich region at the C-terminus in both the majority of vertebrate Dmrt1 genes and the invertebrate mab-3 gene.

Human DMRT1 maps to autosomal locus 9p24.3, the critical deleted region, that, when in monosomy, manifests gonadal dysgenesis and male-to-female sex reversal in XY individuals. Murine Dmrt1 is localized on the central region of chromosome 19 which displays conserved synteny with human distal chromosome 9p. Furthermore, the Z-linked chicken Dmrt1 and human DMRT1 are also at comparable chromosomal locations because of the extensive conserved synteny between chicken Z sex chromosome and human chromosome 9 including 9p24.3 (Nanda et al. 1999).

However, the strongest evidence for conserved DMRT1/Dmrt1 function in testicular differentiation comes from expression studies. In all of the examined vertebrates, DMRT1/Dmrt1 appears to have a gonad-specific and sexually dimorphic expression profile during embryogenesis. However, the same studies have also revealed some differences in the spatial and temporal expression of DMRT1/Dmrt1 gene derived from various vertebrate species. In most of the cases it is up-regulated either late during sex-determination or during the early testis-differentiation period. Thus there is little doubt that DMRT1/Dmrt1 is a versatile vertebrate locus involved exclusively in early male gonad formation.

Dmrt1 as the sex-determining gene

The discovery of the conserved role of DMRT1/Dmrt1 in vertebrate testis development allows for the consideration of DMRT1/Dmrt1 as a switch sex-determining gene, especially in animals not containing Sry gene. This presumption seems to be true in reptiles employing a temperature-dependent sex determining strategy and to chicks with a genetic ZZ/ZW sex-determining mechanism. Dmrt1 is the earliest known gene whose expression is considerably higher in the genital ridge of developing male gonads than in the undifferentiated ovary in these vertebrates. In three of the examined reptilian species with temperature-dependent sex determination, Alligator mississippiensis, Trexenumys scripta, Lepidochelys olivacea, the mRNA level of Dmrt1 was higher in embryos incubated in a male-producing temperature than in embryos incubated in a female-producing temperature (Smith et al. 1999, Kettlewell et al. 2000, Torres-Maldonado et al. 2002). Zarkower suggests that temperature-sensitive expression of Dmrt1 in reptiles may be regulated either by an unknown temperature-depending genetic factor or by itself in the process of autoregulation (Zarkower 2001). The second postulate is more consistent with the primary sex determining function of Dmrt1 in these vertebrate species. In addition, studies in chicken embryos indicate this role for Dmrt1 in male development (Raymond et al. 1999b, Smith et al. 1999, Shan et al. 2000). Expression of avian orthologues becomes sexually dimorphic before the onset of sex differentiation, stronger in developing male than in female gonads. What is more, in the later stages of embryonic development, ovarian Dmrt1 expression becomes progressively weaker and in consequence declines, in contrast to the successive and exclusively higher testicular Dmrt1 expression continued in adult ZZ gonads. These results can be explained by the facts that chicken Dmrt1 maps to the Z sex chromosome and, unlike the mammalian X chromosome in females, there is an absence of dosage compensation in birds. Thus, this avian putative upstream regulatory sex-determining factor seems to be involved in a dosage-sensitive mechanism. The overexpression of Dmrt1 from two Z chromosomes in the genital ridge at the time of sex determination may be required to initiate testis differentiation, whereas one gene dosage in the bipotential ZW gonads is likely insufficient for testis formation and leads to female development. However, recent studies shed doubt on dosage dependence for Dmrt1 in chicken because of the increased level of Dmrt1 expression during experimentally induced female-to-male sex reversal, despite the presence of only one Z chromosome in genetically female gonads (Smith et al. 2003). Nevertheless,
the involvement of a DMRT1 dosage-sensitive mechanism in testis differentiation has also been proposed in humans with defective testis development and sex reversal in XY individuals with monosomic deletion of 9p, which may be due to haploinsufficiency for expression of this male regulatory factor (either by itself or with nearby genes) (RAYMOND et al. 1999a). Furthermore, the expression profile of DMRT1 in developing human embryos is similar to the role of Dmrt1 genes in human and avian sex development. DMRT1 expression, together with SRY, was detected in the genital ridge of 6-week old male embryos and becomes sexually dimorphic (upregulated in adult testis, similar to the testis-specific expression of Dmrt1 genes) in human and avian sex development. Although in chicken both developing male and female gonads express Dmrt1 at a higher level in ZZ embryos, expression of DMRT1 appears not to be detectable in undifferentiated human female embryos at all. Some questions have to appear in the light of these results: does this male-specific gonadal expression pattern of human DMRT1 occur in other mammalian species which either contain or lack SRY? Does chicken Dmrt1 play this same active role in all birds and reptiles with a ZZ/ZW sex-determining mechanism? What is the function of Dmrt1 homologues in lower vertebrates, do they express Dmrt1 in a male-specific manner? Only some of these questions have been resolved so far.

Dmrt1 as a sex-differentiating gene

Although the conserved function of DMRT1/Dmrt1 during mammalian sex development was demonstrated in functional studies generating transgenic mice with positive expression being under the control of porcine DMRT1 promoter sequences (BOYER et al. 2002), other research has indicated some divergence. Unlike the gonadal expression profile of human DMRT1, the murine homologue is expressed in the genital ridge of both sexes at a similar level just before gonadal differentiation and becomes sexually dimorphic (upregulated in testis) after the activation of the Sry gene (RAYMOND et al. 1999b; SMITH et al. 1999; DE GRANDI et al. 2000). This is consistent with the role of Dmrt1 in male gonad differentiation rather than sex determination. Furthermore, male Dmrt1 knockout mice were found to have postnatal affecting Sertoli and germ cells (in some cases similar to the phenotype of 9p deletion patients), but not sex reversal (RAYMOND et al. 2000). This single reported functional data also suggests the redundant function of Dmrt1 in ovary development due to fully fertile Dmrt1+ female murine mutants. This well-evidenced role of Dmrt1 as a male-promoting gene was also shown in lower vertebrates. Investigation of expression profiles on both mRNA and protein levels in the frog Rana arvalis showed the implication of amphibian homologue in testicular, but not ovarian differentiation (SHIBATA et al. 2002; AOYAMA et al. 2003). Expression was restricted only to the developing and adult testis and to gonads in sex-reversed animals obtained by testosterone injection into female tadpoles. Expression of vertebrate Dmrt1 genes in a male-specific manner was also documented in teleost fish, the tilapia Oreochromis niloticus (GUAN et al. 2000), rainbow trout Oncorhynchus mykiss (MARCHAND et al. 2000) and zebrafish Danio rerio (GUO et al. 2005). Although the expression of the rtDmrt1 gene (rainbow trout Dmrt1) was higher in the differentiating testis when compared to the differentiating ovary and occurs in the early stages of spermatogenesis in adult testis, similar to the testis-specific expression of tDmrt1 (tilapia Dmrt1), in both of these fish species there is some association of DM domain genes with ovary development. Unlike all other examined vertebrate DMRT1/Dmrt1 genes, expression of rtDmrt1 was detected in adult female gonads. What is more, the isolation of an additional DM domain gene from tilapia, tDMO (tilapia DM-domain gene in Ovary), expression of which is limited to the ovary, is the first report of a female specific DM domain gene in vertebrates. Additionally, the Dmrt1 orthologue isolated from the lizard Calotes versicolor (a reptile with non temperature-dependent sex determination) appears to play a double role during embryo development: first in testis differentiation by promoting proliferation and differentiation of pretestioli cells, later in gametogenesis in both testis and ovary by promoting proliferation of germ cells (SREENIVASULU et al. 2002). In contrast to these results, two other simultaneous studies have disclosed the presence of a DM domain gene in the sex-determining region on the Y chromosome (DMY/Dmrt1bY) in the medaka fish karyotype (MATSUDA et al. 2002; NANDA et al. 2002), despite the earlier identified autosomal Dmrt1 locus in this fish species (BRUNNER et al. 2001). The duplicated copy of Dmrt1 found on the Y chromosome in its specific region (Dmrt1bY) in fish exhibiting a genetic sex determining mechanism (XX females, XY males), is a sex-determining gene and represents the non-mammalian vertebrate equivalent of Sry. This probably explains the specific expression pattern of both Dmrt1 and DMY during embryogenesis in medaka fish. Unlike other vertebrate homologues, Dmrt1 in O. latipes is expressed after testicular
differentiation and seems to be essential for regulation of spermatogenesis, whereas DMRT1 expression occurs during male gonad differentiation and in an as yet unknown manner acquires the function of a sex-determining gene (KOBAYASHI et al. 2004).

In conclusion, the presented data reveal the functional diversity of DMRT1/Dmrt1 in the large group of vertebrate animals, especially in the extensively examined fish. However, its role seems to be limited to the upstream or downstream testis regulators acting during animal embryogenesis. In the field of sexual development, DMRT1/Dmrt1 still remains the most conserved throughout vertebrates, both in its structure and function.

References


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